1: Biochim Biophys Acta. 1997 Jan 21;1344(2):139-52. Related Articles, Link

Characterization of human apolipoprotein A-I expressed in Escherichia coli.

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Human apolipoprotein A-I (apoA-I), with an additional N-terminal extension (Met-Arg-Gly-Ser-(His)6-Met) (His-apoA-I), has been produced in Escherichia coli with a final yield after purification of 10 mg protein/1 of culture medium. We have characterized the conformation and structural properties of His-apoA-I in lipid-free form, and in reconstituted lipoproteins containing two apoA-I per particle (Lp2A-I) by both immunochemical and physicochemical techniques. The lipid-free forms of the two proteins present very similar secondary structure and stability, and have also very similar kinetics of association with dimyristoyl phosphatidylcholine. His-apoA-I and native apoA-I can be complexed with 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC) to form similar, stable, either discoidal or spherical (sonicated) Lp2A-I particles. Lipid-bound native apoA-I and His-apoA-I showed very similar alpha-helical content (69% and 66%, respectivel in discoidal Lp2A-I and 54% and 51%, respectively in spherical Lp2A-I). Th conformation of His-apoA-I in lipid-free form and in discoidal or spherical Lp2A-I has also been shown to be similar to native apoA-I by immunochemical measurements using 13 monoclonal antibodies recognizing distinct apoA-I epitopes. In the free protein and in reconstituted Lp2A-I, the N-terminal has no effect on the affinity of any of the monoclonal antibodies and minimal effect on immunoreactivity values. Small differences in the exposure of some apoA-I epitopes are evident on discoidal particles, while no difference is apparent in the expression of any epitope of apoA-I on spherical Lp2A-I. The presence of the N-terminal extension also has no effect on the reaction of LCAT with the discoidal Lp2A-I or on the ability of complexes to promote cholesterol efflux from fibroblasts in culture. In conclusion, we show that His-apoA-I expressed in E. coli exhibits similar physicochemical properties to native apoA-I and is also identical to the native protein in its ability to interact with phospholipids and to promote cholesterol esterification and cellular cholesterol efflux.